THIOL METABOLISM IN THE PARASITIC NEMATODE *HAEMONCHUS CONTORTUS*

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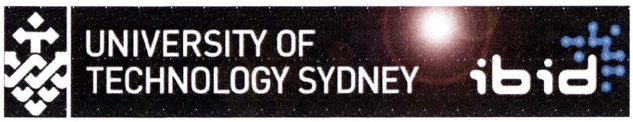
PhD

2010



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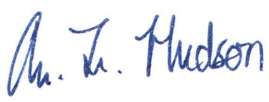

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ACKNOWLEGMENTS

Unquestionably, the person who deserves the biggest thanks is my supervisor Mary Davey. Without you Mary this would not have been possible. Thank you for all your guidance, support, encouragement, friendship and particularly your patience. I know it has not been easy for you having one final student to get rid of, but we did it!

I would also like to give a special thanks to Nick Smith for taking me in when I was offically orphaned and allowing me to become an adopted member of the apicompadres.

To all the students and staff of IBID and MMB, thank you for making it a pleasure to come to work every day. In particular, I would like to thank my fellow 'team meat' members Catherine James and Irene Sotirchos. Without your friendship and guidance I would not have made it through so many years (and still be fairly sane!). Thank you to all my fellow office buddies as well, especially Rowan for putting up with the constant questions, and, Rob, Mike, Alana and Pip for making life entertaining.

I would also like to thank my family and friends, particularly Benjamin Dougall who has had to put up with the 'many moods' associated with being a PhD student.

Special thanks go to John Dalton and Colin Stack for providing the *Hc*Prx2 plasmid and the antiserum.

Finally I would like to acknowledge the funding given by Meat and Livestock Australia, without which this work would not have been possible, and also the conference funding provided by IBID, the science faculty and the Vice Chancellor.

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ABSTRACT

Haemonchus contortus is an important parasitic nematode, both economically and pathologically. The emergence of widespread drug resistance requires new drug or vaccine targets to be identified. The requirement of aerobic organisms to control damage caused by reactive oxygen species and, the increased necessity of parasites to overcome the host immune response, has led to the investigation of antioxidant systems as potential targets. This work examines the thioredoxin antioxidant system in *H. contortus,* specifically the thioredoxin reductase and peroxiredoxin enzymes, to characterise their activity and determine if they are potential targets for parasite control.

H. contortus contains two TrxRs, a cytoplasmic enzyme *Hc*TrxR1 with a selenocysteine in the active site, similar to the mammalian TrxR, and a mitochondrial enzyme *Hc*TrxR2 with a nematode unique active site. *Hc*TrxR1 showed broad activity with thioredoxins from *E. coli*, sheep, and *H. contortus* while *Hc*TrxR2 had high activity with only the mitochondrial *H. contortus* thioredoxin 1. Importantly, *Hc*TrxR1 was found to be more sensitive to the black tea inhibitor theaflavin than the selenocysteine containing mammalian TrxR, demonstrating the differences in the enzymes susceptibilities to inhibitors. To determine the function of the TrxR enzymes in nematodes, knockout (KO) strains of *Caenorhabditis elegans* were examined. TrxR1 -/- KO worms were more sensitive to free radical attack and also to the anthelmintic ivermectin; while TrxR2 -/- KO eggs were highly sensitive to sodium hypochlorite. This demonstrates that inhibition of these enzymes would sensitise the nematodes to the host's immune attack.

H. contortus contains two peroxiredoxins, the mitochondrial *Hc*Prx1 and the cytoplasmic *Hc*Prx2. The activity of both peroxiredoxins was specific for the thioredoxin system; however, both peroxiredoxins were also able to be regenerated by the glutathione system when coupled to the nematode specific *H. contortus* thioredoxin 5. Both enzymes were stable to high concentrations of hydrogen peroxide

which demonstrates different functions to their mammalian counterparts. A specific inhibitor of these peroxiredoxins was also identified which has minimal mammalian cytotoxicity. *Hc*Prx1 was found to be involved in drug resistance while *Hc*Prx2 was found to be secreted and highly immunogenic. Analysis of homologous genes in *C. elegans* showed that both peroxiredoxin KO worms were sensitive to free radical attack; however, only the cytoplasmic *Ce*Prx2 KO *C. elegans* were sensitive to external oxidants.

Overall, this work adds to the knowledge of *H. contortus* biology and identifies the enzymes of the thioredoxin system as potential drug or vaccine targets for parasite control.

JOURNAL PUBLICATIONS

Hudson, A.L., Sotirchos, I.M. and Davey, M.W. (2010) Substrate specificity of the mitochondrial thioredoxin reductase of the parasitic nematode *Haemonchus contortus. Parasitol Res, In Press.*

James, C.E., Hudson, A.L. and Davey, M.W. (2009) An update on P-glycoprotein and drug resistance in *Schistosoma mansoni. Trends Parasitol,* 25: 538-9.

James, C.E., Hudson, A.L. and Davey, M.W. (2009) Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends Parasitol,* 25:328-35.

Sotirchos, I.M., Hudson, A.L., Ellis, J. and Davey, M.W. (2009) A unique thioredoxin of the parasitic nematode *Haemonchus contortus* with glutaredoxin activity. *Free Radic Biol Med,* 46:579-85.

Sotirchos, I.M., Hudson, A.L., Ellis, J. and Davey, M.W. (2008) Thioredoxins of a parasitic nematode: comparison of the 16- and 12-kDa thioredoxins from *Haemonchus contortus. Free Radic Biol Med,* 44: 2026-33.

PRESENTATIONS

Hudson, A.L. and Davey, M.W. (2009) Antioxidants as drug targets for the control of *Haemonchus contortus,* WAAVP, Calgary, Canada.

Hudson, A.L. and Davey, M.W. (2009) A strategy to control parasitic nematodes: The antioxidant system as a drug target, ASP, Sydney, Australia.

Hudson, A.L. and Davey, M.W. (2008) Thiol metabolism and drug resistance in *Haemonchus contortus,* ASP, Adelaide, Australia.

Hudson, A.L., Stack, C., Dalton, J. and Davey, M.W. (2007) Peroxiredoxin, a thiol dependent drug target for *Haemonchus contortus* control, ASP, Canberra, Australia.

Hudson, A.L., Stack, C., Dalton, J. and Davey, M.W. (2007) Peroxiredoxin, a thiol dependent drug target for *Haemonchus contortus* control, MLA research meeting, North Sydney, Australia.

LIST OF ABBREVIATIONS

Abbreviation	Full name
ATP	adenosine triphosphate
BCIP	5-bromo-4-chloro-3-indolyphophate
BLAST	basic local alignment search tool
Bp	base pair
BSA	bovine serum albumin
°C	degrees Celsius
Cys	cysteine
CDNB	1-chloro-2,4-dinitrobezene
cDNA	complementary DNA
ddH_2O	double-distilled water
DMSO	dimethyl sulfoxide
dpy	dumpy phenptype
dsRNA	double stranded RNA
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DTNB	5,5-dithiobis(2-nitrobenzoic acid)
DTT	dithiolthreitol
E	efficiency
EDTA	ethylene diamine tetra acetic acid
ELISA	enzyme-linked immunosorbent assay
ES	excretory-secretory
EtOH	ethanol
FA	Formaldehyde agarose
FAD	flavin adenine dinucleotide
g	centrifugal force (gravity)
G	gauge

GR	glutathione reductase
Grx	glutaredoxin
GSH	reduced glutathione
GSSG	oxidised glutathione
GPx	glutathione peroxidase
Hrs	hour
IC_{50}	inhibitory concentration (50%)
Ig	immunoglobulin
IPTG	isopropyl-β-thiogalactopyranoside
IVF	ivermectin resistant *H. contortus* strain
IVM	ivermectin
Kb	kilobases
K_{cat}	catalytic constant
kDa/K	kilodaltons
K_i	inhibitory constant
Km	Michaelis constant
KO	knockout
L1	first larval stage
L2	second larval stage
L3	third larval stage
L4	fourth larval stage
LB	Luria broth base
Lon	Longer phenotype
m	metres
M	molar
MES	2-(N-morpholino) ethanesulfonic acid
MFO	mixed function oxidase
mins	minutes

MOF	moxidectin resistant *H. contortus* strain
MOPS	3-(N-morpholino) propanesulfonic acid
M_r	molecular weight
mRNA	messenger RNA
MSG	monosodium glutamate
MTT	3-(4,5 dimethylthiazolyl-2)-2,5-diphenyl tetrazolium
MWCO	molecular weight cut off
NADH	beta-nicotinamide adenine dinucleotide
NADPH	beta-nicotinamide adenine dinucleotide phosphate reduced form tetrasodium
NBT	nitro blue tetrazolium
ng	nanograms
NGM	nematode growth media
nm	nanometre
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDI	protein disulfide isomerase
PFC	4-phenyl-3-furoxan carbonitrile
pI	isoelectric point
PMSF	phenyl methane sulphonyl fluoride
Prx	peroxiredoxin
qRT-PCR	quantitative reverse transcriptase PCR
QS	Quackenbush
RACE PCR	random amplification cDNA polymerase chain reaction
RNA	ribonucleic acid

RNAi	RNA interference
ROS	reactive oxygen species
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
SECIS	selenocysteine insertion sequence
secs	seconds
SeCys/U	selenocysteine
SDS	sodium dodecyl sulphate
siRNA	smaller interfering RNAs
SOD	superoxide dismutase
TBE	Tris borate buffer with EDTA
TF	theaflavin
TFBI/II	transformation buffer I/II
TGFβ	transforming growth factor beta
TGR	thioredoxin glutathione reductase
T_m	melting temperature
TNB	2-nitro-5-thiobenzoate
Trx	thioredoxin
TrxR	thioredoxin reductase
Try/TXN	trypanothione
TryR	trypanothione reductase
UV	ultraviolet
V	Volts

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